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Induced Formation of Chelating Agents by Pseudomonas aeruginosa Grown in Presence of Thorium and Uranium[1]

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The aim of this program is to (1) identify microorganisms which bioaccumulate strategically important metals such as chromium, cobalt, niobium, tin and platinum; (2) learn about the mechanisms which govern the bioaccumulation processes at the molecular level, including the chemical characterization of naturally occurring chelating agents; (3) immobilize isolated reactive centers, either cellular or molecular onto an inert carrier so that specific metals can be selectively recovered, and (4) suitably scale up the process developed from

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20. Abstract

the information obtained in (1) - (3). If advisable in the future phases of the program, the most successful organisms will be subjected to genetic engineering manipulation. In the initial phase of this program, laboratory studies are focusing on the metal bioaccumulation properties of whole cell, cell wall preparations and exo-cellular products characteristic to several selected microorganisms. The selected microorganisms are part of a Brookhaven National Laboratory collection which includes strains of metal resistant organisms such as Pseudomonas aeruginosa CSU, P. aeruginosa PAO-1, Saccharomyces cerevisiae, Aspergillus niger, P. fluorescens, Escherichia coli, and Thiobacillus ferroxidans. Interaction of these microorganisms with salts of chromium, tin, manganese, cobalt, platinum, uranium and thorium has been investigated. Cell wall accumulation studies have shown that P. aeruginosa CSU has a preference for uranium while P. aeruginosa PAO-1, Aspergillus niger and P. fluorescens exhibits a preference for thorium under identical experimental conditions. P. aeruginosa CSU cellular biomass when exposed to a mixture of cobalt, chromium and manganese exhibits a preference for chromium. Aspergillus niger under identical conditions is chromium and manganese selective. P. aeruginosa when grown in the presence of high concentration of thorium, produces several chelating agents which can be isolated from the medium. Thorium induced products are not found in the media in which the microorganisms have been grown in the absence of thorium. It has also been shown that P. aeruginosa PAO-1 under identical experimental conditions, when grown in the presence of uranium, responds differently than when grown in the presence of thorium. Two of the thorium induced compounds have been identified as pyochelin and pyriamine. Preliminary results also indicate that some of the bacterially produced exo-cellular compounds resemble, but are not identical, with the known iron chelating agents. For details of the above described work, see enclosed papers (Premuzic et al., 1985; Premuzic and Lin, BNL 36548, 1985; and Premuzic et al., BNL 36301R, 1985). Current trust in the experimental protocol is a study of the interaction between the same organisms used in described studies, and geothermal brines and a laboratory prepared representative mixture. Results of these studies will be described in another report.

Induced Formation of Chelating Agents by *Pseudomonas aeruginosa* Grown in Presence of Thorium and Uranium[1]

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Abstract. Chelating agents produced by microorganisms enhance the dissolution of iron and increase its mobility and bioavailability. Since there are some similarities in the biological behavior of ferric, thorium and uranuyl ions, microorganisms resistant to thorium and uranium and capable of growing in their presence may produce sequestering agents for these metals in a manner similar to those produced for iron. Such complexation would increase the mobility and bioavailability of thorium and uranium in the environment. Pseudomonas aeruginosa species are resistant to certain heavy metals and have also been found in thorium, uranium and plutonium contaminated areas. In the present work the ability of P. aeruginosa to elaborate sequestering agents in medium containing thorium or uranium salts was tested. Addition of 10, 100, and 1000 ppm of uranium or thorium to culture medium increased the lag period of the organism as the concentration of the metal increased. At concentrations of 1000 ppm and higher, there was an extended lag period followed by reduction in growth. Uranium has a stronger inhibitory effect on growth of the organism than thorium at similar concentrations. Analyses of the culture media have shown, that relative to the control, and under the experimental conditions used, the microorganisms have produced several new chelating agents for thorium and uranium.

Some of the bacterially produced compounds resemble, but are not identical to the known iron chelating siderophores isolated from microorganisms, and some of their chemical properties are also discussed.

Introduction

Microbial transformations of toxic metals have been the subject of numerous investigations over the past several years. Such studies deal with microbial resistance to metals, resistance to antibiotics, detoxification mechanisms, biomethylation, bioaccumulation, and microbial solubilization of metals from ores (Varma et al. 1976, Marques et al. 1979, Norris and Kelly 1979, Strandberg et al. 1981). Much is known about autotrophic microbial leaching of metals from coal refuse and ores in acidic environments (Summers and Silver 1978, Wildung et al. 1979, Shumate et al., 1978). However, little is known of the mechanisms and the extent of heterotrophic microbial solubilization and mobilization of toxic metals in organic rich environments, including waste disposal sites.

There is now ample evidence for the existence of biochemical processes, believed to be plasmid mediated (see for example, Summers and Jacoby, 1978, Nakahara et al. 1977), which govern heavy metal interactions with microorganisms.

Studies have shown that heterotrophic microorganisms play a significant role in the transformation and the transport of radionuclides by leaching and formation of complexes with organic matter (Wildung and Garland, 1980, Francis 1982). Organic complexing substances present in soils and natural waters are known to interact with plutonium (Bondietti et al. 1976). The organic substances involved in such interactions are thought to be microbially or plant derived materials (Barnhart et al. 1980, Francis 1982, Premuzic 1983, Blevins et al. 1985). Several aerobic and anaerobic bacteria were isolated from low level radioactive waste disposal sites. They include Bacillus sp., Pseudomonas sp., Citrobacter sp. and Clostridium sp. (Francis,

et al. 1980). A Pseudomonas sp. isolated from a plutonium contaminated pool (Johnson et al. 1974) also bioaccumulated plutonium and uranium (Shumate et al. 1978).

It is well established that microorganisms produce chelating agents by which they bind iron and enhance its solubility and bioavailability in the environment (Neilands 1973, 1977, Winkelmann 1982, Byers et al. 1982, Newsome and Wilhelm 1983, Akers 1983, Vandenbergh et al. 1983). By and large, these chelating agents belong to a group of compounds known as siderophores, structurally cyclic and acyclic small molecular weight compounds (< 1000 daltons), possessing catechol and hydroxamate functional groups (Neilands, 1966, 1967, 1973, 1977, 1979, Raymond 1977, Raymond and Tufano 1982).

It is known that the biological behavior of uranium, thorium and plutonium is similar to that of the ferric iron (Hodge et al. 1973), therefore, it might be reasonable to assume that organisms resistant to such metals as thorium and uranium may produce chelating agents similar but not necessarily identical to those elaborated for iron.

Pseudomonas species already mentioned earlier are a highly versatile and adaptive group of organisms known to elaborate strains possessing resistance to metals and antibiotics (Marques et al. 1979).

In this study we tested the ability of P. aeruginosa PAO-1 and P. aeruginosa CSU, the latter isolated from a plutonium contaminated pond (Johnson et al. 1974), to elaborate chemical compounds capable of complexation with thorium or uranium, and thus influence their transport in the environment.

The experiments had a twofold objective: (1) To establish whether the organisms produced chelating agents during the growth in the presence of

various concentrations of thorium or uranium, and (2) to test whether these oganisms differentiate between thorium and uranium, manifested in the growth pattern and/or the nature and concentration of the natural products (i.e., chelating agents) produced by P. aeruginosa grown in the absence of these metals. Such differences would be indicative of possible specific mechanisms, study of which should ultimately lead to our understanding at the molecular level of the interactions between toxic metals and the resistant species, and in turn, allow us to clarify microbial influences upon the behavior and mobilization of heavy metals such as thorium and uranium, and by analogy, of plutonium, in the environment.

Materials and Methods

Culture

Pseudomonas aeruginosa CSU, known to bioaccumulate uranium (Strandberg et al. 1981) was kindly provided by G. Strandberg, Oak Ridge National Laboratory, TN. P. aeruginosa-PAO-1 obtained from American Type Culture Collection (ATCC 15692).

Culture Medium

Nutrient broth (Diflo, MI) containing 1% dextrose or defined medium (Aickin and Dean 1979) had the following composition per liter: 0.18 mg $FeSO_4 \cdot 7H_2O$; 0.035 mg $MgSO_4 \cdot 7H_2O$; 0.67 g disodium glycerol-2-phosphate hydrate, 0.85 g KNO_3; 1.17 g NH_4NO_3; 2.0 g glucose; 27.1 g trisodium citrate dihydrate; pH adjusted to 6.8 by addition of citric acid (~2 g).

For ATCC 15692, the following medium was used: 5.4 g sodium succinate hexahydrate; 214 mg NH_4Cl; 174 mg K_2SO_4; 81.3 mg $MgCl_2 \times 6H_2O$; 0.137 mg $ZnCl_2$; 0.127 mg $Mn\ Cl_2$; all salts were dissolved in 4 mM potassium phosphate buffered at pH 7.4.

Growth of bacteria was monitored by measuring the absorbance at 600 nm in a Spectronic-20 spectrophotometer. Direct counts of bacteria were determined by acridine orange direct counts (AODC) using epifluorescence microscopy (Hobbie et al. 1977).

Chemicals

Reagent grade thorium nitrate and uranyl nitrate hexahydrate were purchased from ICN Pharmaceuticals, Plainview, NY and were used without further purification. Arsenazo III was purchased from Aldrich Chemical Co., Milwaukee, WI.

Thorium and Uranium Assays

Thorium or uranium in culture medium was determined by a spectrophotometric method using Arsenazo III (Savin 1961, Premuzic et al. 1983). The thorium- or uranium-arsenazo III complex formation is rapid (<1 min) at room temperature, with a lower sensitivity limit of 0.05 ppm for Th and U under experimental conditions used. Since the culture media were slightly colored, difference spectra between thorium containing and thorium free culture media treated in an identical manner were taken throughout the measurements.

Thorium or uranium concentrations in bacterial cell biomass were determined in the following manner. The cells were digested in 7N HNO_3 at 80°C for 24 hr. The acid was evaporated to dryness at 130°C, the residue redissolved in distilled water and Th or U concentrations analyzed by the spectrophotometric method using the arsenazo III procedure.

Ultrafiltration

After the removal of bacterial cells by centrifugation, all supernatant liquids were filtered through a Millipore PTG CO 2570 filter, to remove substances with a molecular weight larger than 5000 daltons. This step

eliminates the larger molecular weight species, e.g., proteins, carbohydrates and polynuclear products (derived from metal ions) which might interfere in isolation and subsequent biological assays.

Characterization of Natural Products Produced by P. aeruginosa in the presence of Thorium and Uranium salts

The products generated by P. aeruginosa in the presence of Th or U, were derived from the following media: (1) uninoculated medium containing 0 ppm Th or U; (2) uninoculated medium containing 100 ppm Th or U; (3) inoculated medium containing 0 ppm Th or U, harvested at time 0 and at late logarithmic growth; (4) inoculated medium containing 100 ppm of Th or U harvested at time 0 and at late logarithmic growth. The media were all centrifuged at 12,000 x g; the supernatants ultrafiltered and stored in sterile containers at 5°C. The chemical composition and properties of the ultrafiltrates were assessed by chromatographic and spectroscopic analyses.

Absorption Spectroscopy

Ultraviolet-visible spectra were obtained with a Beckman Acta-IiI spectrophotometer. Differences in spectral absorption between culture supernatants with and without metal (Th or U) were obtained from: (1) spectra of maximum growth samples run against a reference of zero growth, and (2) spectra of maximum growth samples containing Th or U against a reference of maximum growth without Th or U. Consequently, we could correct and account for the spectral contributions of the medium as well as that of the culture during growth in the absence of metal.

Fluorescence Spectroscopy

Fluorescence spectra of the ultrafiltrates derived from cultures containing Th and U were obtained with a Perkin-Elmer MPF4 fluorescence spectrophotometer by excitation at 360 nm (Cox and Graham, 1979).

Column Chromatography

The ultrafiltrates from culture media were analyzed by gel permeation chromatography. A column (1 x 100 cm) was packed with polyacrylamide gel (Bio-Rad P-2 super fine grade) and calibrated with compounds of known molecular weight: NaN_3, glutathione, and blue dextran. Ultrafiltrates were concentrated by freeze drying. Samples for chromatography were prepared by dissolution of freeze dried material in a minimum amount of water before application to the column. Fractions from elution with deionized water (MilliQ) were monitored at two absorbances, 254 nm and 360 nm.

Thin Layer Chromatography (TLC)

TLC was made on cellulose plates (Brinkman polygram cell 300) and the chromatograms were developed in a mixture of water and isopropanol (1:1, v/v) as the mobile phase. Specific visualization methods and/or reagents (e.g. fluorescence, phenol, and amino groups) were used to detect compounds on the developed chromatograms (Stahl 1969). The colorimetric test for the detection of thorium and uranium, Arsenazo III, was adapted to TLC, and when used in this manner the detection limit was 10 ppm. Arsenazo III reagent does not react with iron. Thorium and uranium complexes were prepared from citrate, oxalate, glucose, and glucose-2 phosphate and were used as standards concomitantly with unknown samples in the TLC runs. The nitrates of thorium and uranium do not migrate under the prescribed experimental conditions. Details for the procedures have been described elsewhere (Premuzic et al. 1983).

High Pressure Liquid Chromatography (HPLC)

Analytical HPLC data were obtained on a IBM LC/9533 liquid chromatograph equipped with an IBM C18 column (No. 8635308). Preparative HPLC data were obtained on an Alltech (No. 6231) C18 preparative column with 50% aqueous methanol as the mobile phase.

Mass Spectroscopy

Mass spectra were obtained on a HP5985 mass spectrometer system which uses electron impact as ion source. Solid samples were introduced directly into the ion source.

Nuclear Magnetic Resonance (NMR)

Proton NMR spectra was obtained on a Varian CFT-20 spectrometer. The solvents used were D_2O and $CDCl_3$.

Effect of Thorium and Uranium Concentrations on the Growth of P. aeruginosa

One ml of a 24-h-old culture of P. aeruginosa was transferred to a 100 ml defined medium containing 0, 1, 10, 100, and 1000 ppm. Th (as $Th(NO_3)_4$). The growth of bacteria was then followed as described earlier. Analogous experiments have been carried cut using defined media containing uranium added as uranyl nitrate.

Effect of Supplemental Iron on the Growth of P. aeruginosa when Grown in the Presence or Absence of Thorium or Uranium

P. aeruginosa was grown in defined medium, (<0.05 ppm in Fe) to which 18 ppm supplemental iron was added as $FeSO_4 \cdot 7H_2O$. In parallel experiments, P. aeruginosa was grown without supplemental iron. P. aeruginosa was grown in the two culture media in the presence and the absence of 100 ppm of Th or U at an initial pH of 6.9. These cultures, in duplicate, were incubated at 30°C on a rotary shaker at 200 rpm and the growth of the

organism monitored. At stationary growth phase the cells were harvested by centrifugation at 12,000 x g, and the supernatants analyzed for Th or U. The cells were dried at 80°C, weighed, digested and analyzed for Th or U. Under the experimental conditions used, the cell mass accumulated less than one per cent (dry weight) of thorium or uranium.

Results and Discussion

Bioavailability of thorium or uranium depends on the speciation of the metal. Speciation is a function of concentration and pH and is particularly important under conditions in which metal ions may undergo hydrolysis and polynuclear formation (Ringbom 1959).

Generally, starting with a near neutral pH, the pH of the culture media became more alkaline, in some cases reaching 8.5, as the growth of the organism progressed, with no apparent effect on the growth.

The effect of the thorium concentration on the growth of P. aeruginosa is shown in Fig. 1. As the concentration of Th in the medium increases, the lag period of the organism also increases. Cultures grew slowly at first and subsequently at a rate nearly equal to that of the thorium free control.

P. aeruginosa, when grown in presence of uranium under identical conditions as those for thorium, behaves differently. Uranium not only increased the lag period, but also had a pronounced effect on growth. For example, at 1,000 ppm of uranium there is a significant inhibitory effect (Fig. 2) not observed with thorium under comparable experimental conditions.

Usually, sublethal concentrations of metals retard the onset of bacterial growth. For example, methylmercury acetate extended the lag phase of cultures of Rhodopseudomonas capsulata, although the cultures which did

begin to grow reached limiting cell densities similar to that of control (Jeffries et al. 1975). Cadmium extended the lag phase of cultures of Escherichia coli, however, normal proliferation was observed at the end of the lag phase (Mitra et al. 1975). During the lag phase, 95% of cells lost viability, and various structural abnormalities were observed, but by the middle of the lag phase, cells had resumed normal morphology. Extension of the lag phase by mercuric chloride was also accompanied by a decrease in viability of R. capsulata although the turbidity remained unchanged (Vaituzis et al. 1975). In the case of E. coli (Mitra et al. 1975), it has been suggested that the cells develop some mechanism of molecular accommodation during this phase. This concept is supported by the changes in cellular distribution observed during growth.

In media containing thorium, supplemental iron did not significantly affect the growth of bacteria. This suggests that the traces (<0.05 ppm) of iron present in the mineral salts are sufficient to support the growth of the organism when grown in the thorium containing medium. Analogous experiments with uranium have shown that there was an increase in lag period in media without supplemental iron. The addition of supplemental iron to the medium decreased the lag period in the growth of the organism by about twelve hours. These results, together with concentration effects already described, further indicate that P. aeruginosa when grown under identical conditions behaves differently when grown in the presence of thorium than when grown in the presence of uranium. This difference is also noticeable in the gross spectral properties of the culture media. During growth of P. aeruginosa in defined medium and in the presence of metal, the microorganism produces fluorescent pigments. For thorium pigments, λ_{max} is at 368 nm and

for uranium pigments, at 365 nm. The production of these pigments increases with time of growth. However, under identical conditions, the onset and attainment of high pigment production in thorium containing medium occurs in a significantly shorter period of time than the corresponding pigments formation in the uranium containing medium, as shown in Table 1. These pigments are not formed in cultures grown in the absence of thorium or uranium. Further, excitation of 360 nm of the clear (at maximum growth) culture medium yields a fluorescence spectrum with a λ_{max} at 433 nm for thorium and a λ_{max} at 440 nm for uranium pigments (Fig. 3).

Pseudomonas sp. are known to have produced chelating agents under a variety of growth conditions and the production of these agents has been expressed in terms of the changes in the spectral properties of centrifuged culture media as discussed below. In addition to spectral changes, extracts and fractions derived from the media at maximum growth have been tested for the presence of specific types of compounds, such as hydroxamates and catechols, known to be incorporated in the chemical structures of bacterial chelating agents (Neilands 1973). Thus Pseudomonas GH has produced an amino group positive, fluorescent compound, L-5(2-pyridyl)-2-amino-5-ketopentanoic acid with a λ_{max} = 358 nm (Shiman and Neilands 1965). Pseudomonas fluorescens migula has produced in the culture medium a λ_{max} 435-440 nm fraction, which was ninhydrin and hydroxamate positive (Maurer et al. 1968). The complete molecular structure has not been elucidated. Pseudomonas fluorescens putida produced a yellow-green fluorescent component in an iron-limited medium with λ_{max} 400 nm, shoulders at 460 nm and 540 nm. The structure of the fluorescent component known as Pseudobactin has been elucidated. The molecule is a linear hexapeptide containing hydroxamate and

quinoline moieties (Teintze et al. 1981). Another compound isolated from the medium was colorless and nonfluorescent, called "Pseudobactin A" and differed only in the saturation in the quinoline moiety (Teintze and Leong 1981).

Pseudomonas aeruginosa grown in an iron deficient medium (< 0.1 μM) yielded an ethyl acetate extractable fluorescent compound, λ_{max} at 218, 248, and 310 nm (Cox and Graham 1979). Excitation spectra show maxima at 235, 272, and 352 nm, troughs at 252 and 310 nm, with emission measured at 442 nm. When excited at 352 nm, the emission spectrum had a single maximum at 442 nm. Excitation of the sample at 235 or 272 nm yielded the same single maximum. A structure of this compound has been reported (Cox et al. 1981). This compound belongs to a little known group of compounds known as Pyochelins which contain catechol and phenolate moieties, as well as functional groups containing sulfur and nitrogen.

A Pseudomonas strain has been reported to yield "Compound S" with a MW 500-600 daltons (McCracken and Swinburne 1979). Aqueous solutions were pale yellow λ_{max} 384 nm, and blue-fluorescent. "Compound S" was negative for catechols and positive for hydroxamate and appears to be an analogue of schizokinen and aerobactin (Mullis et al. 1977, Linke et al. 1972).

Spectral changes observed in our work indicate that similar chelating compounds are generated by P. aeruginosa grown in presence of thorium or uranium. The diagnostic usefulness of spectrum characteristics and their sensitivity to changes in the culture medium is also reflected in the difference spectra (see methods) of thorium and uranium cultures when grown with supplemental iron and without it (Fig. 4). The spectroscopic information shows that at maximum growth the production of thorium

"pigments" (λ_{max} 368 nm, see also Table 1) is essentially unaffected by the iron concentration, while the production of uranium "pigments" (λ_{max} 365 nm) is drastically affected. At maximum growth cultures of P. aeruginosa, grown under identical conditions in the absence of thorium or uranium, did not generate these products following the addition of these metals (as nitrates) to the medium. Therefore the 368 nm and 365 nm signals are due to compounds whose production was induced by the presence of thorium or uranium respectively in the culture medium during the entire growth of the organisms. These signals may be due to single compounds or a mixture of several compounds, including chelators for thorium and uranium present in free and complexed forms.

In order to further explore the nature of substances produced in the presence of thorium or uranium, ultrafiltrates of culture supernatants were fractionated by column chromatography which was followed by thin layer chromatography (see methods). Subfractions from column chromatography were pooled according to their approximate molecular weight ranges into three major fractions, A = 2000-1000, B = 1000-300, and C = $\leq$ 300 daltons respectively. Thin layer chromatography of these fractions and controls, consisting of the autoclaved media containing thorium or uranium with and without supplemental iron, inoculated with P. aeruginosa, was carried out as described under methods.

Analyses of the chromatograms showed that in absence of supplemental iron there are no thorium complexes present in fraction A and that there are five thorium complexes present in fraction B. One complex was present in the control, leaving four new complexes in the molecular weight range of 1000-300, whose formation in the culture medium during the growth of the

microorganism was induced by the presence of thorium. Three of these complexes contained fluorescent groups i.e., isoquinoline, phenol and/or catechol as well as amino and/or hydroxamate components, and one lacked the phenol and/or catechol component. The low molecular weight fraction C contained three complexes of which two were present in the control. The remaining thorium complex was amino and/or hydroxamate positive. There was no thorium detected in three other amino and/or hydroxamate and phenol/catechol positive components of the C fraction. Addition of supplemental iron to the cultures produced a very similar pattern, possibly differing in two components present in trace amounts, in the lower molecular weight fraction.

Identical analyses of uranium complexes showed that in the absence of supplemental iron, fraction A contained two complexes, one with amino and/or hydroxamate and phenol/catechol functions and the other with the phenol/catechol function only. Fraction B contained four complexes, two of which were fluorescent, one contained phenol/catechol functions and amino and/or hydroxamate groups, while the other contained only the phenol/catechol function. The remaining two complexes contained amino and/or hydroxamate functions only. Fraction C, contained three complexes, all fluorescent, two contained phenol/catechol and amino and/or hydroxamate functions and one contained phenol/catechol function only.

In the presence of supplemental iron, fraction A contained three complexes, one present in the control, while the others induced by the presence of uranium were fluorescent, contained amino and/or hydroxamate and phenol/catechol functions. One of these based on the R_f value (see Table 2) was different from the complex present in cultures to which supplemental iron

was not added. Fraction B contained five complexes, one of which was also present in the control. The remaining complexes, induced by uranium presence, were all fluorescent, contained amino and/or hydroxamate and phenol/catechol functions. Similarly, fraction C contained three fluorescent, amino and/or hydroxamate and phenol/catechol positive complexes.

The distribution of thorium and uranium induced complexes is summarized in Table 2.

High pressure liquid chromatography (HPLC) of maximum growth cultures in absence of thorium, but to which thorium was added prior to analysis (Fig. 5a) and those which were grown in the presence of thorium (Fig. 5b) also confirmed presence of thorium induced microbial products. Mass spectoscopy and nuclear magnetic resonance of HPLC fractions obtained from the maximum growth cultures of P. aeruginosa, grown in the presence of thorium, yielded the following information.

Chromatography of an acidified ethanol extract of PAO-1 thorium culture yielded several fractions containing compounds with molecular weights ranging from 208 to >600 daltons. Fraction containing M/e 209 is consistent with pyrimine $C_{10}N_2O_3H_{12}$ (Shiman and Neilands 1965), whose identity is further supported by the mass fragmentation pattern of M/e 209, 163, 130, 79, 75, and its PMR spectrum with signals (in ppm) at 3.8, 2.12, 2.33, 8.2, 8.4, 7.5, and 9.04. A fraction containing a compound with molecular weight of $M+H^+325$ with a fragmentation pattern of M/e 325, 223, 220, 219, 191, 178, 146, 137, 120, 102, and 100, is consistent with that reported for a pyochelin, $C_{14}H_{16}N_2O_3S_2$ (Cox et al. 1981). The PMR spectrum with signals at (in ppm) 9.18, 6.85-7.42, 4.93, 4.42, 3.78, 3.29, and 2.65 further confirms the presence of this compound.

The remaining fractions contain analogues of pyochelin and schizokinen whose structures are currently being identified. Preliminary data indicate that at least three fractions contain compounds which do not resemble those reported to be present in Pseudomonas sp. Detailed chemical and structural identification of the thorium induced products as well as analogous studies with uranium will be reported in a separate paper.

The experimental evidence presented in this paper shows that P. aeruginosa grows in the presence of thorium and uranium. Increases in the concentration of thorium increased the lag phase, however the effect of thorium on the growth was not inhibitory, as was the case with uranium, under identical growth conditions. Hence P. aeruginosa exhibits a different growth pattern towards thorium and uranium. Spectroscopic and chromatographic analyses of culture supernatants show that the organism in the presence of metal during its growth produces several induced products. Products generated during the growth in the presence of these metals contain phenol/catechol fluorescent varieties as well as amino and hydroxamate functional groups. Some of these chelating agents resemble those isolated previously from several strains of P. aeruginosa. Such microbially produced natural products by virtue of their chelating capabilities may enhance the mobility and bioavailability of the organic forms of thorium and uranium and by analogy of other toxic metals in the environment.

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Table 1*

Production of Fluorescent Pigments by P. aeruginosa Grown in the Presence of Thorium and Uranium

Time hrs.	Th ppm.	Cell Density (O.D. 600 nm)	Pigment O.D. 368nm	U ppm.	Cell Density (O.D. 600nm)	Pigment O.D.
0	0	0	0	0	0	0
47	0	1.3	0	0	1.02	0
0	100	0	0	100	0	0
29	100	0	0	100	0	0
47	100	0.26	0.04	100	0	0
53	100	0.6	0.05	100	0.12	0
57	100	0.9	0.06	100	0.2	0
71	100	1.47	1.90	100	1.08	0
120	-	-	-	100	1.20	0.72
192	-	-	-	100	1.20	0.60

*For experimental details see text

Table 2*
Distribution of Thorium and Uranium Induced Complexes

		Thorium Complexes				Uranium Complexes			
Fraction	M.W Range	R_f	<0.05 ppm Iron	R_f	18 ppm Suppl. Iron	R_f	<0.05 ppm Iron	R_f	18 ppm Suppl. Iron
	2000								
A	to	-		-		0.60	+	0.56	+
	1000	-		-		-		-	
						0.78	+	0.78	+
	1000	0.53	+	0.53	+	0.53	+	0.49	+
B	to								
	300	0.62	+	-	-	0.62	+	0.57	+
		0.74	+	0.74	+	0.71	+	0.66	+
		0.79	+	-	-	0.78	+	0.78	+
		-		-	-	0.53	+	0.55	+
C	<300	-		0.72	+	0.62	+	0.62	+
		0.78	+	0.78	+	0.78	+	0.78	+

*For experimental details see text

Figure Captions

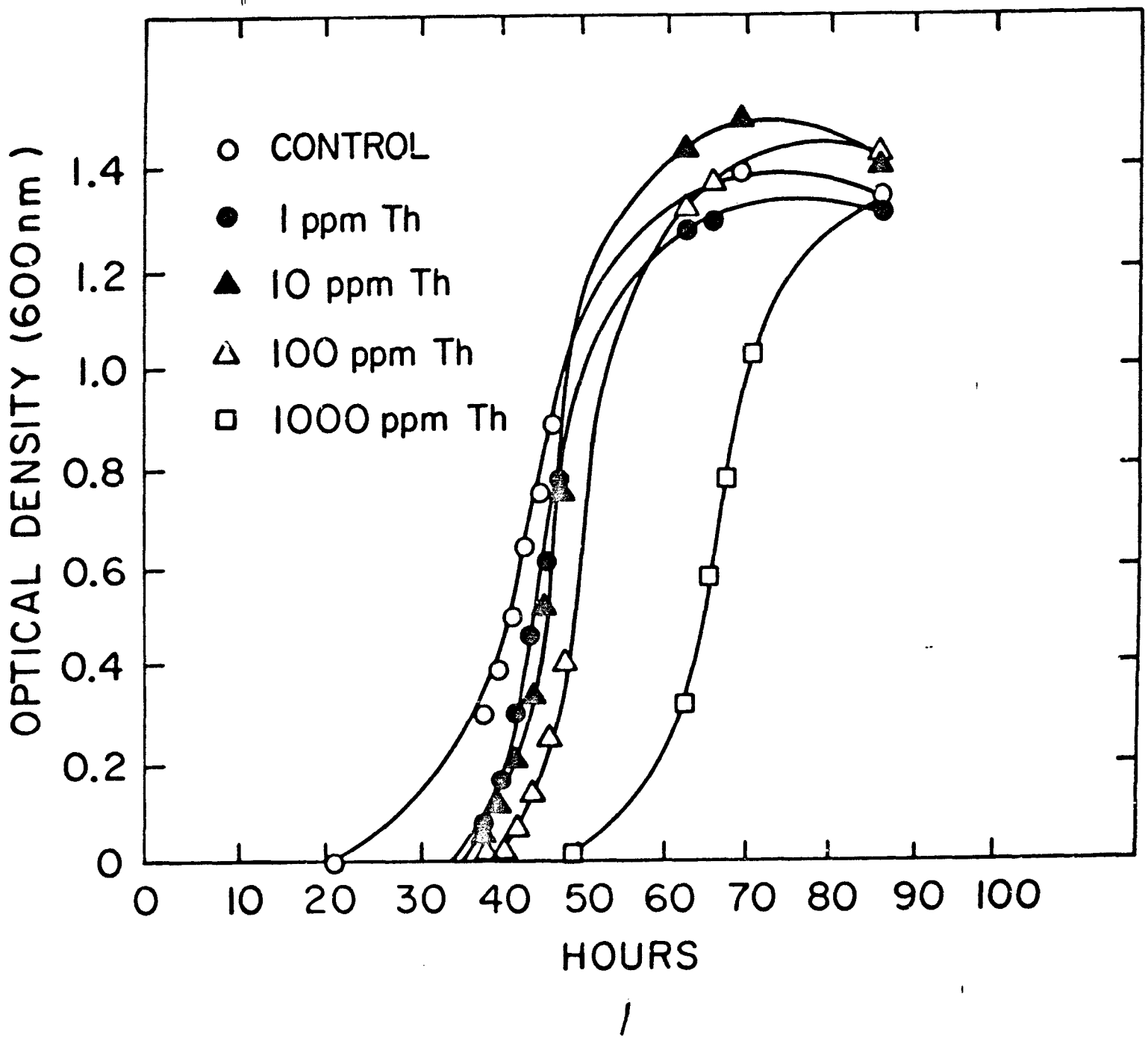
Fig. 1. Effect of various concentrations of thorium on the growth of *P. aeruginosa*.

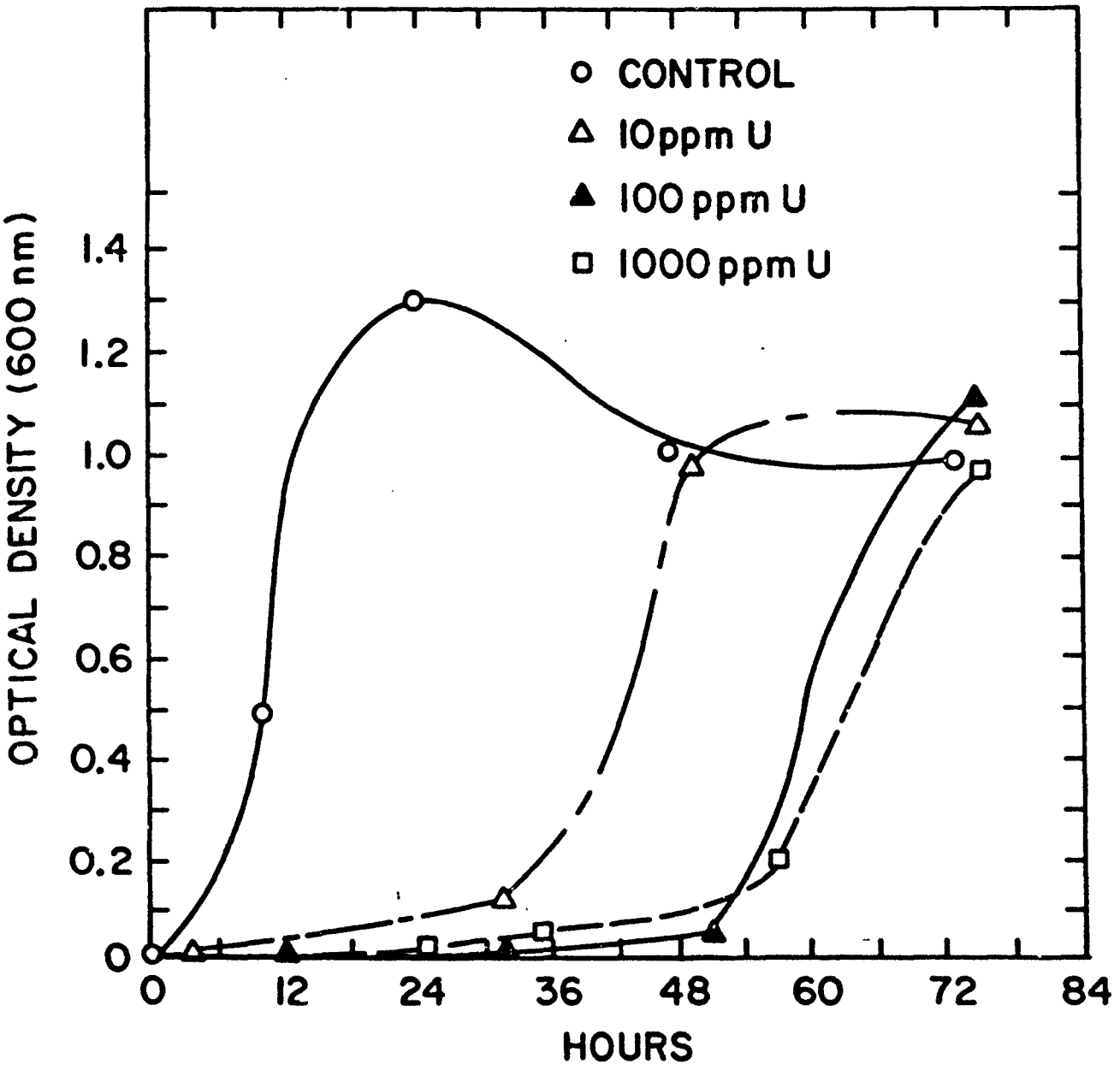
Fig. 2. Effect of various concentrations of uranium on the growth of *P. aeruginosa*.

Fig. 3 Excitation of 360 nm of thorium medium (a) and uranium medium (b).

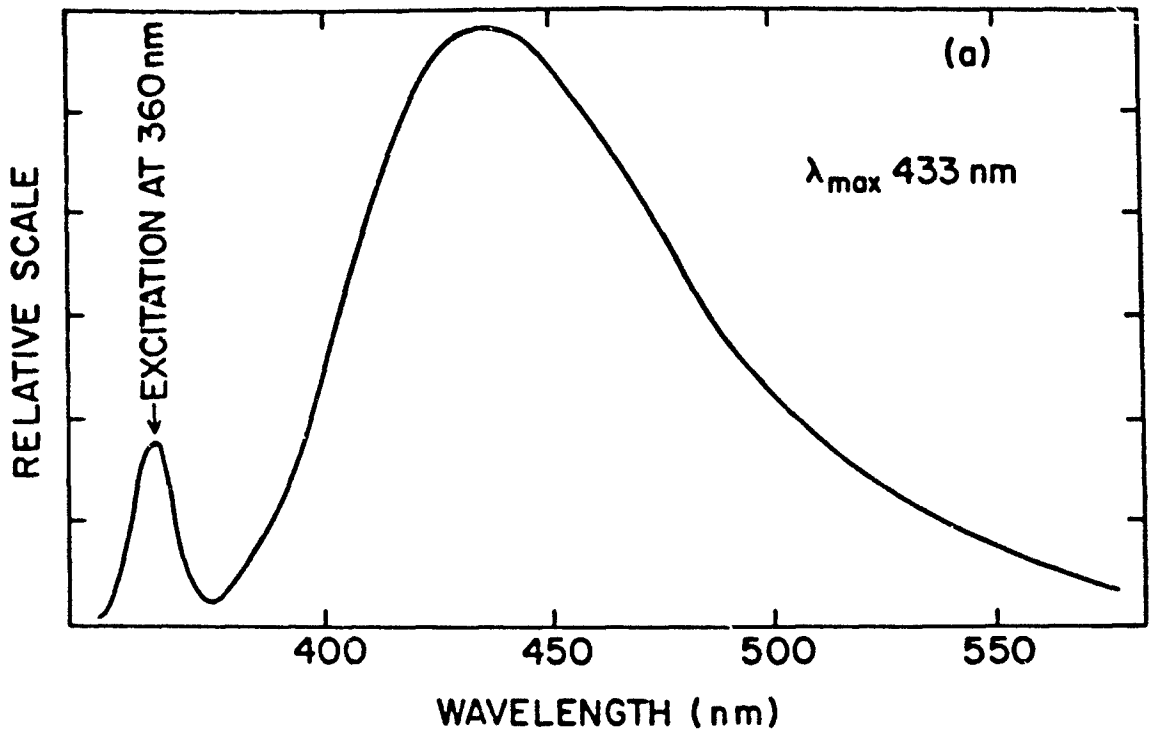
Fig. 4. Effect of supplemental iron on the production of pigments in thorium medium (i) supplemental iron, spectra (a) and uranium medium (ii), supplemental iron spectrom (b).

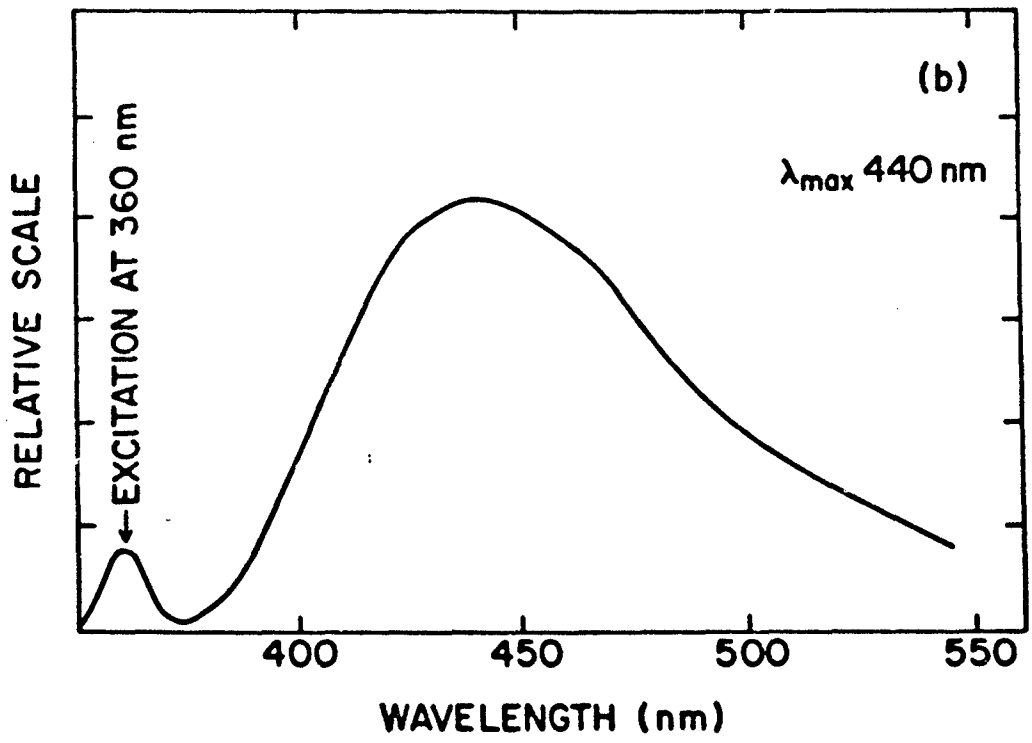
Fig. 5. HPL chromatograms of ultrafiltered supernatant culture media.
(a) Thorium added to the culture medium at maximum growth.
(b) Maximum growth in the presence of thorium in the culture medium throughout the growth.

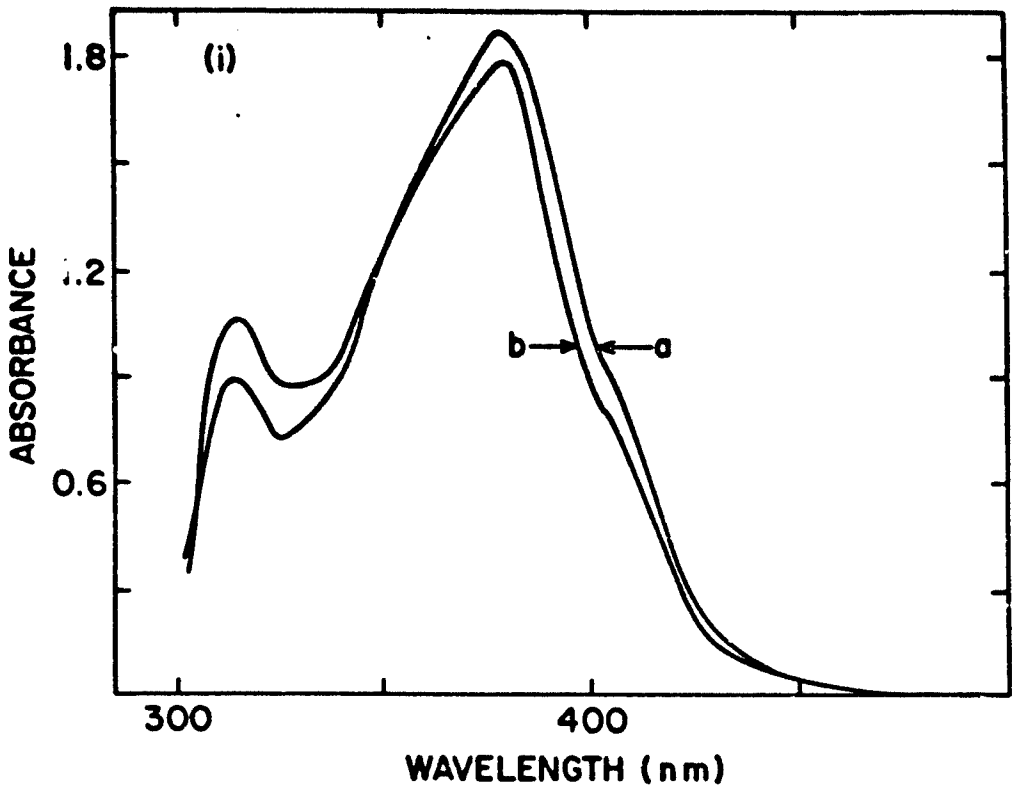

CONTROL
1 ppm Th
10 ppm Th
100 ppm Th
1000 ppm Th
OPTICAL DENSITY (600nm)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
10
20
30
40
50
60
70
80
90
100
HOURS

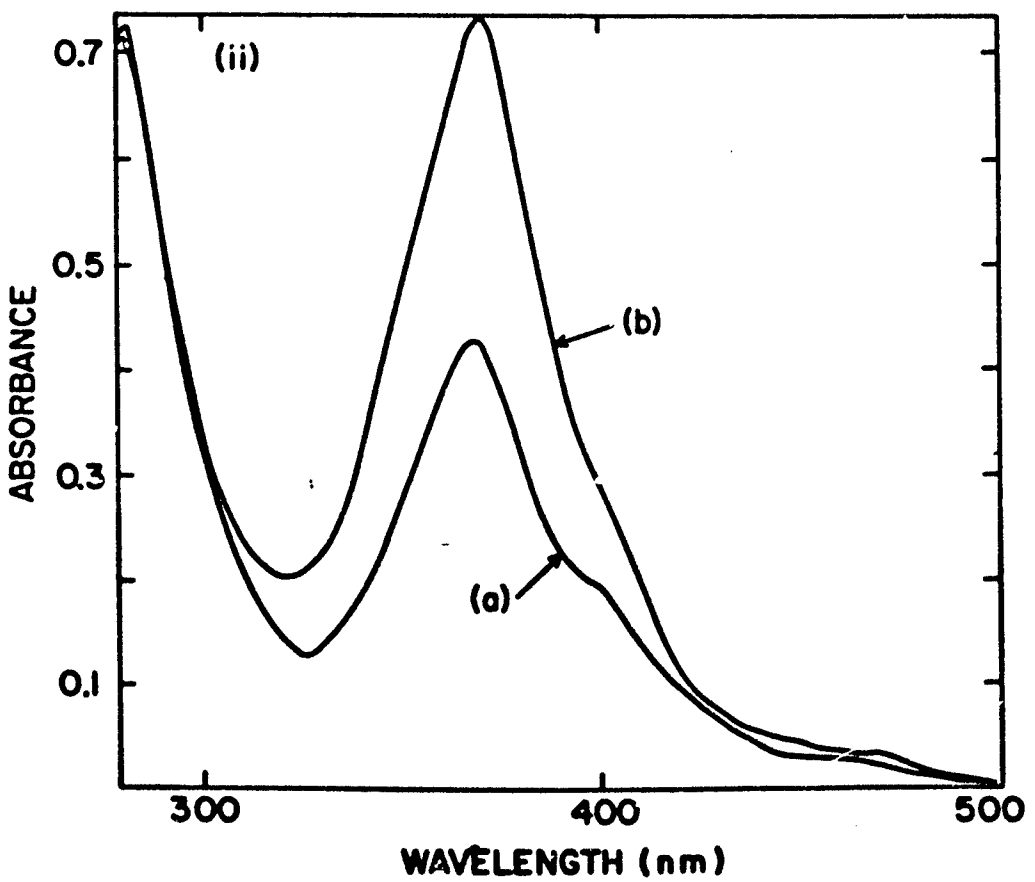

CONTROL
10ppm U
100 ppm U
1000 ppm U
OPTICAL DENSITY (600 nm)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
12
24
36
48
60
72
84
HOURS

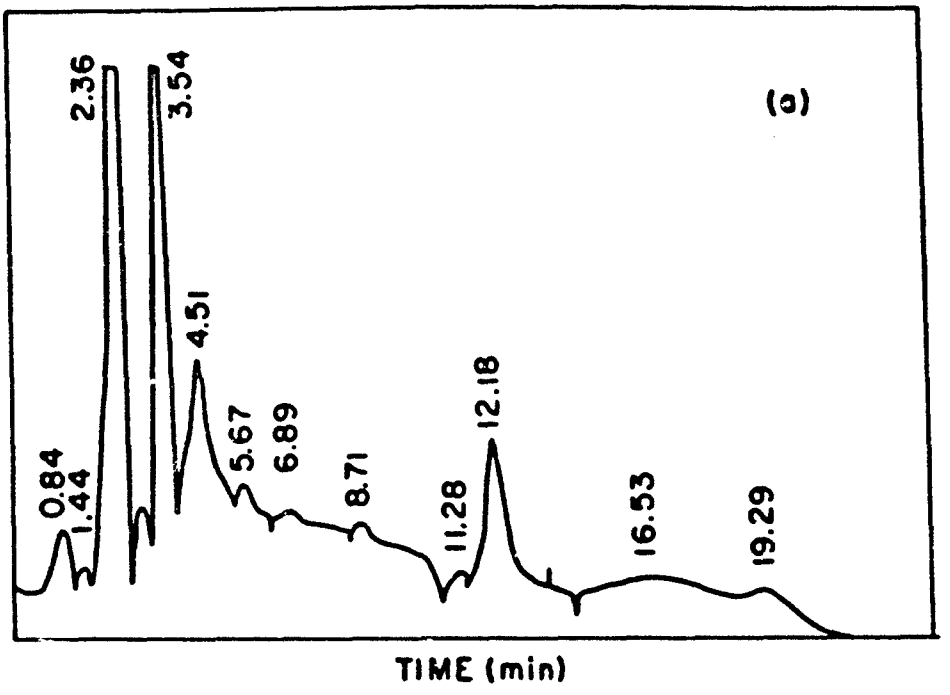
-2

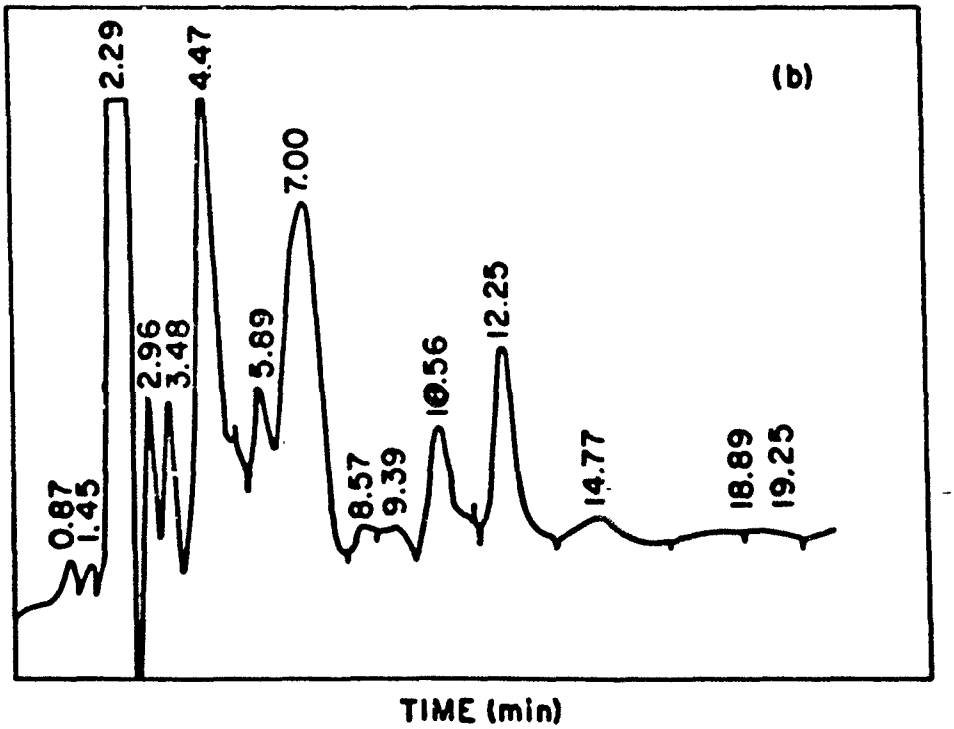

(a)
λ_{max} 433 nm
←EXCITATION AT 360nm
RELATIVE SCALE
400
450
500
550
WAVELENGTH (nm)


(b)
λ_{max} 440 nm
←EXCITATION AT 360 nm
RELATIVE SCALE
400
450
500
550
WAVELENGTH (nm)


(i)
1.8
1.2
0.6
ABSORBANCE
b
a
300
400
WAVELENGTH (nm)


(ii)
0.7
0.5
0.3
0.1
ABSORBANCE
(b)
(a)
300
400
500
WAVELENGTH (nm)


(a)
0.84
1.44
2.36
3.54
4.51
5.67
6.89
8.71
11.28
12.18
16.53
19.29
TIME (min)


(b)
0.87
1.45
2.29
2.96
3.48
4.47
5.89
7.00
8.57
9.39
10.56
12.25
14.77
18.89
19.25
TIME (min)